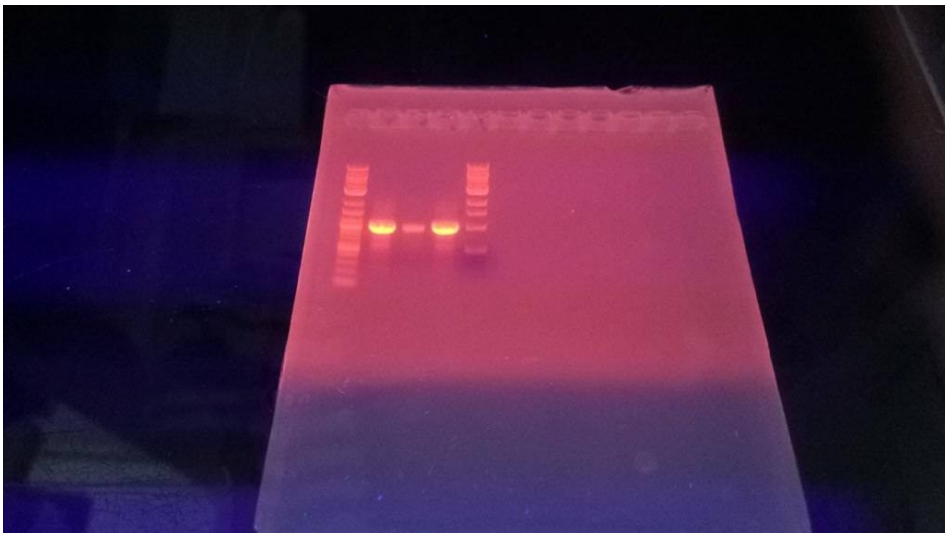
Thursday, June 2, 2016 (Christine Tang, Kevin Chen, Jessica Lee, Margaret Lie)

Objective: To do colony PCR for iRFP713.

1. Followed the Colony PCR procedure (on Benchling).
2. Thermal cycling:

Temperature (Celsius)	Time (minutes)	Cycle
95	0:30	
95	0:30	
57.4	0:30	35X
68	0:30	
68	5:00	
4	∞	

3. Ran gel to confirm size of iRFP713.
    a. Used 0.8% agarose gel.
4. We saw bands at 1 kB for the 3 colonies, suggesting that the iRFP713 inserts are correct. Tomorrow, will miniprep and send them for sequencing.



Friday, June 3, 2016 (Christine Tang, Jessica Lee)

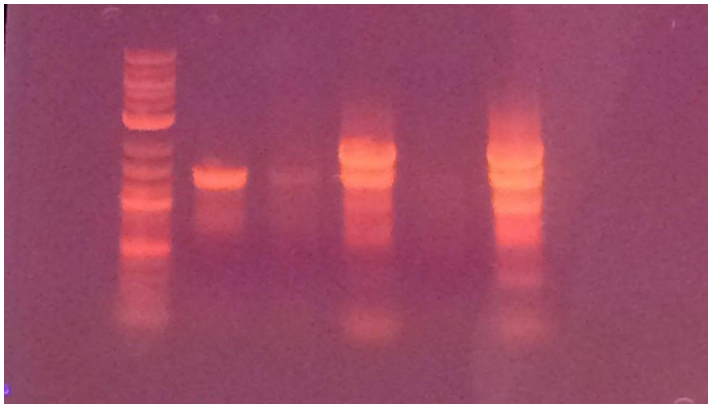
Objective: MIniprep iRFP713 samples from June 2, 2016, then send them for sequencing.

1. Followed QIAprep Spin MIniprep Kit protocol (Hilden, Germany).
2. Used Nanodrop to obtain concentrations.
3. Sent samples to Genewiz for sequencing. Used primer iGEM 3.
    a. Also sent 2 samples (iRFP713) for sequencing that were miniprepped by Jessica in May.
4. Stored the 3 iRFP samples in Plasmids box #1.

Monday, June 6, 2016 (Kevin Chen, Christine Tang, Margaret Lie)

Objective: To do colony PCR for violacein and check sequencing results for iRFP713.

1. Results of sequenced iRFP713.
    a. #1 = bad. #2 = bad. #3 = good. #4 = good. #5 = deletion at 1383.
2. Followed the Colony PCR procedure (on Benching) for violacein.
    a. Insert size ~7200 bp. Used 3:31 minutes for elongation time.
3. Ran gel verification for violacein.
    a. 0.8% agarose gel for 35 minutes at 80 V.
        i. Lane 1: 2 log ladder
        ii. Lane 2: V#1 = band at 1.5 kb
        iii. Lane 3: V#2 = faint band at 1.5 kb
        iv. Lane 4: V#3 = many bands
        v. Lane 5: V#4 = no bands
        vi. Lane 6: V#5 = many bands
    b. None of the bands are at the right size ~7 kb. We will still send V #1,3,5 for sequencing.



Tuesday, June 7, 2016 (Margaret Lie and Christine Tang)

Objective: To miniprep violacein and send them for sequencing. To transform VioABDE and VioABCE for the purpose of obtaining VioC.

1. Miniprepped violacein 1 & 3. Followed QIAprep Spin MIniprep Kit protocol (Hilden, Germany).
2. Nanodropped for concentrations and sent to Genewiz for sequencing.

Label	Sample	Primer
ML01	V1	VF2 (forward)
ML02	V3	VF2
ML03	V5	VF2
ML04	V1	VR (reverse)
ML05	V3	VR
ML06	V5	VR

Wednesday, June 8, 2016 (Christine Tang, Kevin Chen, Margaret Lie)

Objective: To check sequencing results

1. V1 - VF = good
2. V1 - VR = good
3. V3 - VF = bad
4. V3 - VR = good
5. V5 - VF = good
6. VF - VR = good

Friday, June 10, 2016 (Christine Tang, Kevin Chen, Margaret Lie)

Objective: To create 100 µM stocks and 10 µM stocks for primers iGEM 15, 16, 17, and 18. To send iRFP670 for sequencing.

1. Created 100 µM stocks of iGEM 15, 16, 17, and 18, then 10 µM stocks.
2. Sent iRFP670 to Genewiz for sequencing.

Tuesday, June 14, 2016 (Kevin Chen, Margaret Lie)

Objective: To transform electrocompetent (EC) *E. coli* with constitutive promoter, SoxR, terminator and SoxS.

1. Filled four centrifuge tubes with 10 µL of DNAse-free water each.
2. Stabbed wells of iGEM distribution plates containing desired DNA. Transferred water to each well and resuspended DNA until a red color emerged from dye. Let sit for at least 5 minutes.
    a. Promoter BBa_J23106 Plate 4 Well 17P
    b. RBS + SoxR Plate 1 Well 21I
    c. Terminator BBa_B0015 Plate 3 Well 3F
    d. SoxS Plate 1 Well 21M
3. Followed Electroporation protocol (on Benchling).
    a.
4. Incubated 100 µL, 1:100 dilution and 1:1000 dilutions plates for 16-18 hours at 37 degrees Celsius.
5. Will colony PCR tomorrow.

Wednesday, June 15, 2016 (Christine Tang, Margaret Lie)

Objective: To analyze the results of yesterday's bacterial transformation and to colony PCR.

1. Took plates out of the incubator at about 10:30 AM and analyzed them. Results:
    a. Undiluted 100 µL plates had many colonies. Promoter BBa_J23106 had fewer colonies when compared to the other three.
    b. 1:100 and 1:1000 were placed back into the incubator until noon. After they were taken out at noon, they still had fewer colonies than the undiluted 100 µL plates.
    c. Pictures of plates on Benchling.
    d. After 12 P.M., 1:100 and 1:1000 plates had fewer colonies than undiluted 100 µL plates.
2. Set up colony PCR
    a. Picked 2 colonies each for the promoter, terminator, SoxR, and SoxS
    b. On LB/Amp plate:
        i. 1, 2 = promoter

ii. 3, 4 = SoxR

c. On LB/Cm plate:
   i. 5, 6 = terminator
   ii. 7, 8 = SoxS

d. Thermocycler Settings:
   i.

95 degrees Celsius	3 min	
95 degrees Celsius	30 s	Repeat 35x
50 degrees Celsius	30 s	Repeat 35x
72 degrees Celsius	30 s	Repeat 35x
72 degrees Celsius	10 min	
4 degrees Celsius	Infinite hold	

   ii.

3. Incubated plates for 18 h at 37 degrees Celsius and placed bacterial cultures incubator at 37 degrees C, 250 rpm for 18 h.

Summary: Checked the plates from yesterday, found that more colonies grew on undiluted plates. Colony PCRed bacteria and will run gel tomorrow. We plan on miniprepping the bacterial cultures tomorrow if the gel looks good. We will also check for bacterial colony growth on plates.

Thursday, June 16, 2016 (Christine Tang)

Objective: Gel verification of transformed promoter, terminator, SoxR, SoxS
Miniprep to send for sequencing.

1. Ran gel for all 8 PCR samples from yesterday.
2. Miniprepped 8 samples.
   a. Centrifuged cultures for 3 min at 8000 rcf.
3. Sent samples for sequencing.
   a. #1 BBa_J23106 promoter = 416.7 ng/µL
   b. #2 BBa_J23106 promoter = 596.8 ng/µL
   c. #3 SoxR = 382.8 ng/µL
   d. #4 SoxR = 189 ng/µL
   e. #5 BBa_B0015 terminator = 250 ng/µL

f. #6 BBa_B0015 terminator = 187.9 ng/µL
g. #7 SoxS promoter = 232.9 ng/µL
h. #8 SoxS promoter = 231 ng/µL

Summary: Gel pictures put on Benchling. All samples sent to GeneWiz for sequencing. We will see if the cloning occurred successfully.

Friday, June 17, 2016 (Christine Tang and Margaret Lie)

Objectives: Dilute primers

1. Created 100 µM of primers iGEM 28, 29, 38, 27, 49, 50, 30, 33, 36, 37, 34, 35, 45, 42, 43, 44, 45, 46, 47, 48.
    a. Note: We ordered 2 #45 - one made by Christine and one made by Kevin
2. Created 10 µM working stocks

Summary: Figure out which iGEM 45 promoter corresponds to which tube. The other primers are ready to use.

Monday, June 20, 2016 (Christine Tang and Margaret Lie)

Objective: Transformation of Promoter BBa_J23106 + SoxR and Terminator BBa_B0015 + SoxS

*Restriction Digests

1. Incubated following tubes for 1 h at 37 degrees Celsius
    a. Tube 1:
        i. 1.2 µL #1 BBa_J23106 promoter (500 ng)
        ii. 2 µL CutSmart Buffer
        iii. 1 µL Spe1-HF
        iv. 1 µL Pst1-HF
        v. 9.8 µL dH_2O
    b. Tube 2:
        i. 2.65 µL #4 SoxR (500 ng)
        ii. 2 µL CutSmart Buffer
        iii. 1 µL Xba1
        iv. 1 µL Pst1-HF
        v. 8.35 µL dH_2O
    c. Tube 3:
        i. 2.66 µL #6 BBa_B0015 terminator(500 ng)

ii. 2 µL CutSmart Buffer
iii. 1 µL Spe1-HF
iv. 1 µL EcoR1-HF
v. 8.34 µL dH_2O

d. Tube 4
i. 2.17 µL #8 SoxS (500 ng)
ii. 2 µL CutSmart Buffer
iii. 1 µL EcoR1-HF
iv. 1 µL Xba1
v. 8.83 µL dH_2O

2. Incubated tubes 1 and 3 at 80 degrees C and tubes 2 and 4 at 65 degrees C for 20 minutes
3. Added 1 microliter Calf Intestinal Phosphatase to tubes 1 and 4. Incubated 1 h at 37 degrees C. Stored tubes 2 and 3 at 4 C.
4. Ran all DNA on 1.5% agarose gel for 30 minutes at 70 V.
5. Cut larger bands for tube 1 and 4. Cut smaller bands for tube 2 and 3
   a. Tube 1 = 2096 bp
   b. Tube 2 = 509 bp
   c. Tube 3 = 152 bp (very faint)
   d. Tube 4 = 2117 bp
6. Purified DNA using Zymo Gel Purification Kit (Irvine, CA). Eluted twice with 6 microliters of water.
7. Nanodropped to find concentration

*Ligation

1. Set up ligations. Incubated at room temperature for 20 minutes
   a. Tube 1:
      i. 100 ng promoter digest = 5 microliter
      ii. 72.9 ng SoxR digest = 5 microliter
      iii. 10X ligase buffer = 2 microliter
      iv. T4 DNA ligase = 1 microliter
      v. dH_2O = 2 microliters
   b. Tube 2:
      i. 100 ng SoxS digest = 4 microliter
      ii. 21.5 ng terminator digest = 3 microliter
      iii. 10X ligase buffer = 2 microliter
      iv. T4 DNA ligase = 1 microliter
      v. dH_2O = 5 microliters
2. Purified DNA with Zymo Clean and Concentrator Kit (Irvine, CA).

*Transformation

1. Electroporated 4 microliters ligation into *E. coli* XL1 electrocompetent.
2. Incubated 1 h at 37 degrees C, 350 rpm.
3. Plated 100 microliters, 1:100, and 1:1000 on LB/Amp(100) and LB/Cm(34).
4. Incubated overnight at 37C.

Summary: Hopefully, the ligation worked correctly.

Wednesday, June 22, 2016 (Jennifer Carson-Gonzalez and Margaret Lie)

1. Took plates out of incubator
    a. 100 microliters = lawn
    b. 1:100, 1:1000 = better
2. Set up colony PCR
    a. Tm = 51.6 degrees Celsius
    b. For promoter + SoxR = 505 kb, 16 s
    c. For terminator + Sox S = 732 kb, 22 s
3. Plated on LB/Amp(100) for promoter combo and on LB/Cm(34) for terminator combo)
4. Ran gel to verify.

Summary: Should have switched the elongation times. Tube 1 should be 22 s and tube 2 should be 16 s. Will miniprep 1, 3, 4, 6, 7, 8 tomorrow at 9 A.M.

Thursday, June 23, 2016 (Jennifer Carson-Gonzalez and Christine Tang)

Objectives: Miniprep 2 Parts of Nitric Oxide Sensor

1. Miniprepped promoter + SoxR 1, 3, 4 and terminator + SoxS 6, 7, 8.
2. Nanodropped samples and sent for sequencing

*Making iRFP 670 Biobrick Compatible

3. PCR pBAD/HisB-iRFP670. Should be in my box with tube labelled 670 (Look in lab notebook to see which ones are go to use).
    a. Primers 17b and 18b
    b. PCR product length: 5040 bp
    c. 58.4 C

Material	Volume	Concentration

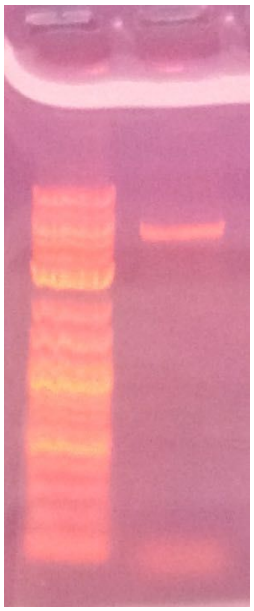
dH20	32.5 QS 50µL	-
5x Phusion HF Buffer	10 µL	-
dNTPs (50x)	1 µL	10 mM
Fwd Primer (20x)	2.5 µL	10 µM
Rev Primer (20x)	2.5 µL	10 µM
Template	Variable (~1uL)	2-10 ng
Phusion DNA Polymerase (100x)	1 µL	100x stock

4. Thermocycling:

Temp. (°C)	Time	Cycle
98	0:30	
98	0:10	
45-72*	0:30	25-35X
72	0:30/kb	
72	10:00	
4	∞	

   a. *Round down Tm for lowest annealing temperature primer

5. Run 5 microliters of PCR product on gel made of 0.8% agarose. 70 V



6. Add 1 microliter Dpn 1 to rest of PCR product. Incubate 1 h at 37C.
7. Zymo Clean and Concentrate. Then check DNA concentration with nanodrop.
8. Analyzed sequencing. All BBa_J61002 + SoxR and BBa_B0015 + SoxS plasmids are functional.

Friday, June 24, 2016 (Rohan Bhardwaj, Margaret Lie, and Christine Tang)

Objectives: Making iRFP 670 BioBrick Compatible

1. Golden Gate DNA Synthesis
    a. **Reaction Setup**

1.43 µl	Largest DNA piece (~100 ng) from PCR product yesterday
1.5 µl	10x NEB T4 Buffer
1.5 µl	10x BSA*
0.5 µl	BsaI
0.5 µl	NEB T4 Ligase, 400.000 cohesive end units/ µl
QS 15 µl	dH2O

    b. *Without the addition of BSA *Bsa*I is only 10% active at 37°C
    c. **Thermo Cycling**

Temp. (°C)	Time	Cycle
37	3:00	
16	4:00	25x
50 (final digestion)	5:00	
80 (heat inactivation)	5:00	

2. Zymo DNA Clean and Concentrate
3. Transform 6 microliters into 1 tube (approx. 40 microliters) of XL1 E. coli by electroporation.
4. Incubate 1 h in 1 mL SOC at 37C, 250 rpm.
5. Plate 100 microliter, 1:100 dilution, and 1:1000 dilution on LB/Amp(100).
6. Incubate at 37C for 16 h.

Summary: Hopefully, will have colonies on the plates.

Saturday, June 25, 2016 (Margaret Lie and Christine Tang)

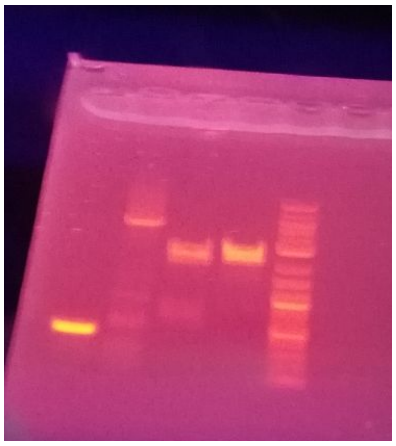
*Transformation of SoxR/S
Title: Transformation of SoxR/S

1. Choosing which plasmids to use:

Look at the different plasmids on benchling. They should already have the sequencing attached. Pick the best ones to use.

Restriction Digests:

2. Put following in tubes.
    a. Tube 1: 500 ng promoter/SoxR + 2 microliters Cutsmart buffer + 1 microliter EcoRI-HF + 1 microliter SpeI-HF + QS to 20 microliter dH2O
    b. Tube 2: 500 ng terminator/SoxS + 2 microliters Cutsmart buffer + 1 microliter XbaI + 1 microliter EcoRI-HF+ QS to 20 microliter dH2O
3. Incubate tubes 1 h at 37 C. After 1 h incubation,
    a. Incubate tube 1 at 80 C for 20 minutes
    b. Incubate tube 2 at 65 C for 20 minutes
4. Add 1 microliter Calf Intestinal Phosphatase to tube 2. Incubate at 37 C for 1 h. Store tube 1 at 4C.
5. Run gel. 0.8 % agarose.



6. Cut out larger bands for tube 2. Cut smaller band for tube 1.
    a. Look up size.
    b. Tube 1: 547 bp, Unwanted = 2056 bp
    c. Tube 2: 2252 bp, Unwanted = 15 bp
7. Purify DNA using Zymo Gel Purification Kit.
8. Nanodrop DNA to find concentration.

Ligation:

9. Ligation

Tube 1 = 73 ng

Tube 2 = 100 ng

varies µL	Vector (1 moles)

varies µL	Insert (3 moles)
1 µL	T4 DNA Ligase
1.5 µL	10x Ligase Buffer
QS to 15µL	dH2O

10. Incubate @ RT for 20min
11. If using electroporation: Zymo purify digestions,

To calculate molar ratio (Generally use 3 moles insert : 1 mole vector, with ~100ng of vector DNA)
http://www.insilico.uni-duesseldorf.de/Lig_Input.html

Transformation:
12. Electroporate ligation.
13. Incubate 1 h at 37C, 250 rpm in 1 mL SOC. (See recipe on another sheet)
14. Plate on LB/Chl (34).

Summary: Hopefully, we will have colonies tomorrow.

Sunday, June 26, 2016 (Margaret Lie)

*Hypoxia iRFP 670

1. Set up PCR
   i.

Material	Volume	Concentration
dH20	37.5 QS 50µL	-
ThermoPol Buffer	5 µL	-
dNTPs (50x)	1 µL	10 mM
iGEM 15 (20x)	2.5 µL	10 µM
iGEM 16 (20x)	2.5 µL	10 µM
Template (ML BBa_K239006 iRFP 670)	Variable (~0.5uL)	2-10 ng
VentR DNA Polymerase (100x)	1 µL	100x stock

   b. Thermocycling:

Temp. (°C)	Time	Cycle
95	5:00	
95	0:30	
59.5	0:30	25-35X
72	1:07	

72	10:00	
4	∞	

2. To prepare gBlock (template)
    a. Centrifuged 5 s at least 3000 xg.
    b. Added 100 microliters dH2O.
    c. Vortexed briefly.
    d. Incubated at 50 degrees Celsius for 20 minutes.
    e. Vortexed briefly and centrifuged.

Summary: Will run gel, Dpn1 digest, Zymo Clean and Concentrate, Nanodrop, etc. tomorrow. Need to take out Biobrick compatible iRFP 713 plate between 11:30 A.M. and 1:30 P.M.

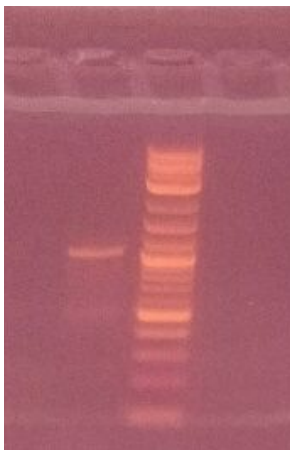
Monday, June 27, 2016- Wednesday, June 29, 2016 (Christine Tang, Jennifer Carson-Gonzalez, Peter Suzuki)

Objectives: Gel Verification of Hypoxia iRFP 670, violacein vector

*Take out plate = Biobrick compatible iRFP 713 from 37C. Parafilm. Store at 4C.

*PCR of hypoxia and iRFP 670 (Second thermal cycler from left - tube named hypo 670)

1. Set aside 5 microliters.
    a. Add 1 microliter of 6X loading dye.
    b. Run for 30 minutes on 1.5% agarose gel at 70-80 V.
2. Take a picture of gel.



3. If correct size = 1110 bp, Zymo Clean and Concentrate DNA after adding Dpn1 and incubating (see step 2).
    a. If incorrect size, run the rest of the DNA on a new gel and cut out the band at 1110 bp and use the Zymo Gel Purification Kit.

4. Add 1 microliter Dpn1 to remaining 45 microliters of PCR product. Incubate 1 h at 37C.
5. Zymo Clean and Concentrate
6. Nanodrop DNA

*Digest Set Up

1. Set up digestions.

Tube 1:

x µL	hypoxia iRFP gBlock 500 ng
2 µL	CutSmart Buffer (in Stratacooler)
1 µL	SpeI-HF
1 µL	EcoRI-HF (10U)
QS 20 µL	dH2O

Tube 2:

x µL	violacein vector or pSB1C3 linearized backbone -> See the last box in my stack. You will have to reuspend backbone so ask for help. 500 ng
2 µL	CutSmart Buffer
1 µL	SpeI-HF
1 µL	EcoRI-HF (10U)
QS 20 µL	dH2O

2. Incubate at 37C for 1-2hr. Then heat inactivate at 80C for 20 minutes.
3. Run on 0.8 % agarose gel.
4. Cut 1.1 kb band for hypoxia.
5. Cut 2047 bp band for violacein vector.
6. Extract (remember to use Saran wrap) and use Zymo Gel Purification Kit.

1. Nano drop.

*Ligation

1. Set Up

varies µL	Vector pSB1C3 (1 moles)
varies µL	Insert (3 moles)
1 µL	T4 DNA Ligase
1.5 µL	10x Ligase Buffer
QS to 15µL	dH2O

2. Incubate @ RT for 20min

3. If using electroporation: Zymo purify digestions,

To calculate molar ratio (Generally use 3 moles insert : 1 mole vector, with ~100ng of vector DNA)

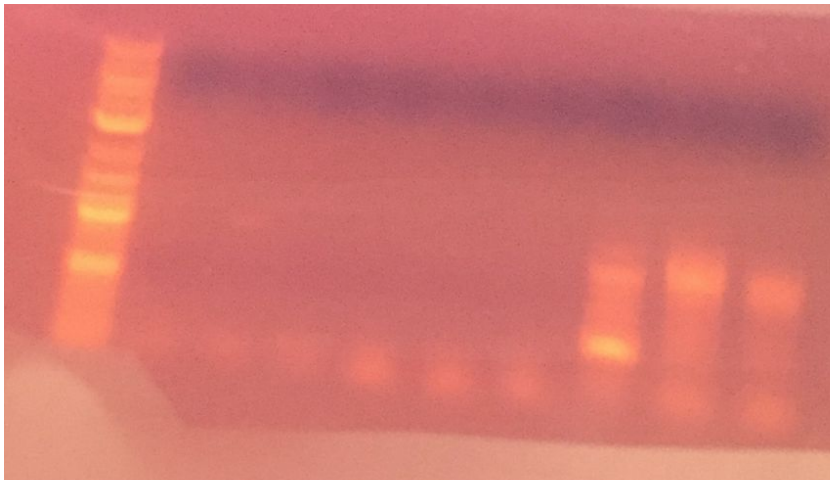
http://www.insilico.uni-duesseldorf.de/Lig_Input.html

*TRansformation

1. Transform by electroporation into XL1EC.
2. Incubate 1 h at 37 C in 1 mL SOC.
3. Plate 1:100 and 1:1000 dilutions on LB/Chl(34) plates.

*Colony PCR Set Up

1. Set up colony PCR for biobrick compatible 670, 713, and SoxR/S.

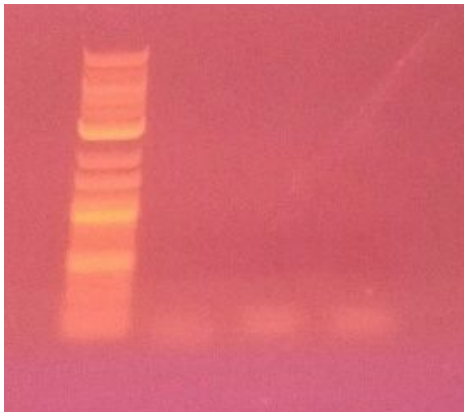


Well 1: 2log ladder; Samples: SoxR/S 1-3; 670 4-6; 713 7-9

Thursday, June 30, 2016 (Peter Suzuki and Christine Tang)

Gel Verification of the 3 Hypoxia/iRFP670/Violacein ligation PCR samples, Miniprep then Send for Sequencing

1. Gel verification of the 3 PCR samples (Hypoxia, iRFP670 and Vio) using a 0.8% agarose gel.



2. Miniprepped all 3 samples, nanodropped and sent for sequencing.

Summary: Gel did not confirm anything, but we miniprepped and sent for sequencing. Check lab notebook for concentrations